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证　明

本证明之附件是向本局提交的下列专利申请副本

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造 名 称：增效抗疟药复方本芴醇的制备方法

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1991 年 5 月 20 日

4.根据权利要求1所述，一种增效抗疟药复方的制备方法其特征在于该复方是由1—10份重量的本芴醇（Ⅰ）与1份重量结构式（Ⅱ）中的一个化合物组合而成。

5.根据权利要求1所述，一种增效抗疟药复方的制备奋发，其特征在于该复方是由3—7份重量的本芴醇（Ⅰ）与1份重量结构式（Ⅱ）中的一个化合物组合而成。

6.根据权利要求1所述，一种增效抗疟药复方的制备方法，其特征在于该复方是由1份重量的本芴醇（Ⅰ）于5—6份重量的蒿甲醚（Ⅱ）组合而成。

7.根据权利要求1所述，一种增效抗疟药复方的制备方法，其特征在于该复方是在加固体添加剂的情况下，将其有效成分配制成片剂。

说　明　书

增效抗疟药复方本芴醇的制备方法

本法明涉及一种增效抗疟药复方和复方治疗疟疾的给药方案以及制备该复方的方法。

耐药性的疟疾是临床和公共卫生的严重问题，而恶性疟原虫 (Plasmodium falciparum)已借基因突变或适应能力对标准抗疟药的氯喹产生了耐药性.这种耐氯喹及其它抗疟药的恶性疟原虫的蔓延和传播是热带及亚热带地区卫生保健工作的主要难题,因此,需要一种能成功治疗耐药性恶性疟的新药或复方.

增效抗疟药复方中本芴醇 (benflumetal)单药的抗疟作用在《化学文摘》中已有报道（Chemical Abstracts 97:28538 h 和101:136941）由其它已知抗疟药组成的复方，例如，氨酚喹(amodiaquine)和四环素(teracycline)复方已用与临床[Suphat Noeypatimanond等(1983)，用氨酚喹和四环素复方在泰国中部治疗恶性疟，Trans.R.Soc.Trop.Med.and Hyg.73(3),338-340]。新近，另一抗疟复方Fansimef [甲氟喹 (mefloguine)，乙胺嘧啶 (Pyrimethamine) 和周效磺胺 (Sulnhadoxine)] 正处于临床试用阶段 ("Tropical Deseuses Research, Seventh Programme Report", Chapter 2; Haluria, UNDP WORLD BANK/WHO, Published by WHO 1985)。

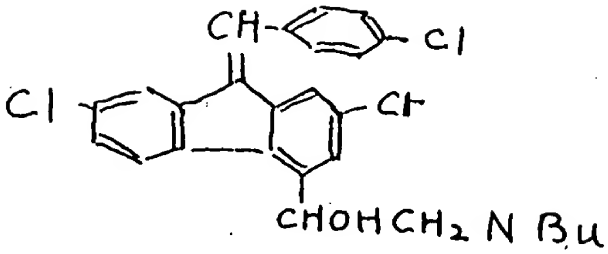
青蒿素(artemisinine)及其衍生物与其它抗疟药，如奎宁，组成的复方已在印度申请专利26BOM/87　和在德国申请专利p3715378。　青蒿素与伯氨喹 (Primaquine) 伍用的增效作用也见诸报道 (Wan Yaode, Cang Qizhong, Pharmacy Bulletin Vol,16,Nol,1981)。

抗疟药青蒿素、蒿甲醚（artemether）蒿乙醚 (arteether) 二氢青蒿素 (dihydroartemisinine)或青蒿琥酯(artesunate)与奎尼定(guinidine)与甲氟喹组成的复方已在欧洲申请的专利362810终公开报道。

本发明的目的在于为临床治疗疟疾的需要提供一种具有疗效高，并能降低恶性疟原虫耐药性的抗疟药复方。

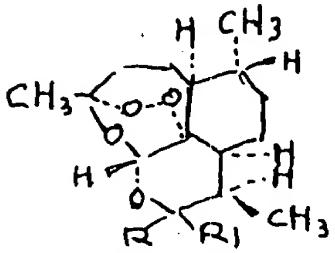
本发明所提出的抗疟药复方，包含本芴醇与青蒿素类药物的组合，尤其是与青蒿素衍生物的疗效更为显著。

本发明设计的抗疟药增效复方是由本芴醇（结构式 I ）的增效剂量。



（Ⅰ）

与青蒿素衍生物（结构式Ⅱ）中至少一种化合物的增效剂量组成。



（Ⅱ）

其中R和R1共同代表氧或R 代表羟基，C1-C6-烷氧基，C1-C6-烯氧基，C1-C5烷酰氧基羧基—C1-C6-烷酰氧基，环已羧氧基，苯甲酰氧基或萘酰氧基；R1代表氢。

该复方由上述抗疟有效成分与药学上可接受的添加剂配制而成的适当剂型。

本发明说明书中使用的定义和术语一般含义如下：

药物复方(Pharmaceutical Composition)是指由本芴醇结构式（Ⅰ）和结构式（Ⅱ）中至少一种化合物组成的混合物。该混合物或为抗疟有效成分（Ⅰ）和（Ⅱ）的干燥制剂，如冷冻干燥剂，或最好为含有适当添加剂以制成各种不同的剂型，如片剂胶囊或栓剂。

“增效作用”一词的定义是复方的药效至少高于含一种成分的药效，更好的是能提高复方中各种成分的疗效，而最理想的增效作用是伍用各种组成成分的低剂量便能提高或/和改善药物的疗效。本发明保护权项的增效作用，已被体外和体内实验所证实，此等实验结果表明：复方中本芴醇（成分Ⅰ）的疗效高于使用单药的疗效；复方中蒿甲醚Ⅱ）的疗效也高于蒿甲醚单独使用的疗效。

根据本发明有抗疟增效作用，所以当服用一种剂型进行治疗时，如每日口服1或2种片剂，也可以联合使用不同的药物治疗方案。

复方制剂中的有效成分本芴醇（Ⅰ）使复方具有杀灭疟原虫彻底的作用，而

复方中的第二种成分（II）如蒿甲醚（R、R1分别代表OCH3和H），对疟原虫则有速效。这些都已被体内及体外药理学实验所证实。

有效成分（I）命名为本芴醇，式中Bu表示正一丁基（见C.A.R.N 82186-77-4）含本芴醇单药制剂的抗疟作用已有报道（见C,A.97:28538 h 和101:13694 i）本芴醇的制备方法已公开于中国专利申请公报中88/07666.X。

有效成分（II），其中R和R1共同代表氧，命名为青蒿素；R或R1分别代表羟基和氢，命名为二氢青蒿素。

在化合物结构式（II）的一个化合物中，C1-C6-烷氧基最好代表甲氧基或乙氧基。化合物（II）中R和R1分别代表甲氧基和氢，命名为蒿甲醚；R 和R1分别代表乙氧基和氢，命名为蒿乙醚。

在结构式（II）的化合物中，C1-C6烯氧基最好是烯丙氧基，C1-C5烷酰氧基最好是乙酰氧基或丙酰氧基，羧基—C1-C6-烷酰氧基—正—丙酰氧基，羧基可以盐的形式存在（羧酸盐），如钠盐或甲盐。化合物（II），R和R1分别代表正—丙酰氧基羧酸钠（$-O-CO-CH_2CH_2CH_2COONa$）和氢，命名为青蒿琥酯。

本发明说明书中所用的有关名称来自 "Tropical Diseases Research Seventh Programme Report}. Chapter 2: Malaria UNDP, WORLD BANK/WHO Published by WHO。

有效成分（II）中青蒿素，二氢青蒿素、蒿乙醚，蒿甲醚以及青蒿琥酯均为已知化合物。青蒿素是从黄花蒿（Artemisia annua L.）中分离得到，并已人工合成，已用于治疗恶性疟（H.P.Koch(1981),Qinghaosu:A Potent antimalaria/from plant origin,pharmacy International (New Drugs).P184-185,Elsevier Nortn Holland Biomedical Press;L.J.Bruce-Schwart(1982),Qinghaosu:a new antimalarial,British Med.J.,184,767-768]。Kocb于1985年曾报告用青蒿素治疗2069例疟疾病人，其中包括1511例间日疟病人的临床评价 [H.P.Kocb(1981). Qingghaosu: A Potent antimalarial from Plaut Origin, Pharmacy International (New Drugs),P.184-185,Elsevier North Holland Biomedical Press]。青蒿素对人的抗氯喹恶性疟原虫株有效 (J. P. Jiung et al(1982), Antimalaria activity of mefloquine and quighosu. Lancet ii 8293, 185-187]。二氢青蒿素，蒿乙醚，蒿甲醚和青蒿琥酯均为半合成的青蒿素衍生物，它们的抗疟活性已在世界卫生组织的各种报告资料中报露 [WHO, Report of the

Scientific working Group of the Chemotherapy Malaria, TDR/Chemal 3rd Review,85,3,Geneva,3.5,June 1985及其所包括的参考文献]。

根据本发明药物复方中最好含有药学上可接受的添加剂。按照常规配制方法，这些添加剂可用于制备经肠道或非肠道吸收的各种剂型。

供口服用的合适添加剂包含惰性填料稀释剂，从而制成各种剂型，例如片剂、粉剂、胶囊剂及类似剂型。如有需要，药物复方中还可以增添其它的成分，如调味剂，粘和剂和赋形剂等类成分。

例如片剂可以含各种固体添加剂，如淀粉、糊精，藻酸和某些复合硅酸盐，以及粘合剂，如聚乙烯吡咯烷酮，蔗糖，明胶和阿拉伯胶等。另外，润滑剂，如硬酯酸镁，十二烷基硫酸钠以及滑石粉常用作片剂的制造。相似类型的固体成分也可以作为软胶囊或硬胶囊的填料，常用的填料有乳糖以及高分子量的多聚乙二醇类。

其它的口服剂型可将药物混合物装在明胶胶囊中服用，还可用合适的精制食用油，如葵花籽油、玉米油、花生油、椰子油或芝麻油等为基础，进行配制。

本发明的具体实施是将有效成分（Ⅰ）和（Ⅱ）配制成单一的剂型单元，如片剂或胶囊剂。

本复方的活性成分（Ⅰ）和（Ⅱ）可分别制成单药制剂，置于同一包装内（多组分药盒），同时服用或先后服用。这两种单药制剂可采用同一服药途径服下，也可以先服组分（Ⅰ）的片剂或胶囊，相继再服用组分（Ⅱ）的制剂。各个给药方案可在临床治疗病人时形成，例如在疟疾首次发作后先服一次大剂量或多次小剂量的组分（Ⅰ）的片剂或胶囊，同时服用小剂量的组分（Ⅱ）的片剂和胶囊，在治疗的过程中再服低剂量的组分（Ⅰ）和高剂量的组分（Ⅱ）。在同一个药盒中，组分（Ⅰ）和组分（Ⅱ）的剂型也可以不同，例如组分（Ⅰ）是片剂而组分（Ⅱ）是栓剂，二种组分可以同时用也可相继使用剂量范围可根据剂型而变化。

本发明抗疟药复方的实用性已为所确立的体内和体外实验结果所证实，有些结果将在实例中介绍。本复方对伯氏鼠疟原虫的高度耐药虫株仍具有高效和速效反映出本发明的实用性。

本发明的权项应包括使用上述由活性组分（Ⅰ）和（Ⅱ）所组成的复方治疗疟疾现症病人的治疗方法。病人服复方的疗程至少是4（3）天，最好是5天或更

长一些。

“治疗方法”一词也包括预防服药，以保护健康人在疟疾高发区，特别是位于山羊星座和蟹星座之间的热带地区，不发疟疾。

复方中活性（Ⅰ）的剂量，可视患者的病情及病程而变动，其变动的范围可以很广。根据伯氏原虫/小鼠模型体内实验结果（将在实例中报告），本芴醇的可用剂量为0.2-5.0mg/kg，0.2-10.0mg/kg更好。由于本芴醇的毒性低，耐受性高，该药的剂量还可根据需要，作相当幅度的提高。实验测得复方中的活性组分（Ⅱ）的可用剂量，蒿甲醚为0.2-5.0mg/kg，0.3-3.0mg/kg更好，特别是0.4-5.0mg/kg更为适宜。

复方中组分（Ⅰ）与（Ⅱ）之间的剂量比例，也可以在相当大的范围内变动。实验测得组分（Ⅰ）的剂量（按重量计）等于或低于组分（Ⅱ）时都可有增效作用，因此，组分（Ⅰ）与（Ⅱ）之间的重量比，可在1：1到10：1之间变动，特别是当组分（Ⅱ）为蒿甲醚时，每服一份蒿甲醚即服3—7份本芴醇，特别是5—6份本芴醇更为适宜。

本发明还涉及及增效复方的制备方法，将结构式（Ⅰ）中一个化合物的有效剂量进行组合，再与药学上可接受的添加剂配制成适合的剂型。

本发明药物复方中的有效成分含量应为10%—80%，以20%—60%为最适含量。根据本发明药物复方适合与经肠道给药，宜配制成口服单元剂型，例如糖衣药丸，片剂，胶囊剂和栓剂等这些剂型可按常规制备工艺配制如采用混合、制粒，成型，溶解或冷冻干燥等工序。例如口服剂型的制备可将有效成分与固体载体混合，再制成颗粒。在混合制粒过程中，如果需要加入适当的添加剂以制成片剂或糖衣丸（片）心。

具体的配制过程，将有效成分（Ⅰ）和（Ⅱ）分别或一起研磨成大约10u—400u最好是20u-200u大小的粒子，至少90%的有效成分粒子应在这个范围内。

这样大小的粒子可用常规的粉碎方法获得，即在空气喷射磨，球磨微粉化最好用Brandon Sonifier型的超声粉碎机(g.Pharm. Sei,53(9):1040-1045(1965))或用高速搅拌机(Homonex型，由Brogli andco.Basel提供)搅拌混悬物，可用500-10,000 rpm转速的搅拌将复方中的有效成分溶解或悬浮于有机溶媒，如甲醇、乙醇、丙二醇中，然后在大约0-5℃用水或2%氯化钠(Nacl)溶液中沉淀，则得微晶(也可以在Nacl溶液中加入低浓度0.1-1%的保护胶体，如明胶和纤维素醚，如甲

基纤维素或羟丙基甲基纤维素）。将混悬物过滤，滤饼与低温（约0－5℃）和减压（50微巴以下，最好0.5微巴以下）干燥。随后可在50－90℃干燥。

将上述获得的晶体（微晶）再制成颗粒，最好按标准的湿法制粒。

制粒时，将药物过筛（必要时粉碎），并与赋形剂（也可不加）混合，用其它的溶媒，如乙醇和水，使颗粒紧密，然后去除溶剂，干燥，再添加（也可不加）润滑剂或助流剂，如硬脂酸镁或吐温，将制成的颗粒搅拌后再过筛一遍。

将制成的颗粒用常规压片机，如柯氏偏心制片机（EKO）Korsch eccentric tabletting machine），以10KN压力压制成片心，再用溶解或分散有聚乙二醇和蔗糖的含水乙醇溶液进行片剂包衣。

药片（或片丸）心可用含阿拉伯胶，滑石粉，聚乙烯吡咯烷酮或聚乙二醇的浓蔗糖溶液进行包衣，从而可以耐胃液的破坏作用。在片剂和药丸糖衣外可涂布着色剂或色素，以示区别或指示含不同剂量的活性成分。

此外，供口服用的药物复方可以是干填充的明胶胶囊，也可以是含颗粒型有效成分与填料乳糖、粘合剂、淀粉和/或助流剂滑石或硬脂酸镁以及稳定剂的掺合物，软胶囊中的有效成分最好是溶解或悬浮适当的液体中，如脂肪油类，石蜡油或液态多聚乙二醇类，也可以加入稳定剂。

适合于肠道给药的复方剂型还有栓剂，由复方的有效成分与栓剂基质组成。作为栓剂基质的有天然的或人工合成的甘油三脂类，石蜡类，多聚乙二醇类或高级烷醇类。也可采用由复方的有效成分与基础材料组成的明胶直肠用胶囊，合适的基础材料有液态的甘油三酯类，多聚乙二醇类或石蜡碳氢化合物。

以下的实施例是说明本发明的上述情况，但并不意味着限制本发明的范围。

实施例1：

本芴醇和蒿甲醚两药组方的剂量比例测定：

a，以伯氏疟原虫感染小白鼠，利用正交设计试验，按照“4天抑制法试验”进行复方的不同剂量平行对照试验，其ED_{50}或ED_{90}是采用直线回归方程计算法测定的，增效指数按下式计算：

$$\text{增效指数} = \frac{\text{单药成分的 } ED_{50}\text{或}ED_{90}}{\text{复方中相应成分的 } ED_{50}\text{或}ED_{90}}$$

按此方程式计算出复方抗鼠疟的伯氏原虫的最佳剂量比例为2:0.75,ED_{90}的增效指数＞6。

b,进一步使用诺氏疟原虫（P.KNOWLESI）感染恒河猴，实验结果显示抗疟复方中本芴醇与蒿甲醚之间的最适剂量比例（按重量计）为3—6:1。

实施例2:

复方本芴醇和蒿甲醚两个成分之间产生的增效作用是根据Peters方法测定的(Peters:Am,Trop.Med.Parasitol Vol.62.Pg488-492(1968))。其结果如下表:

伯氏疟原虫K173株感染的小白鼠在“4天抑制试验中”，给与口服不同比例的蒿甲醚（A）和本芴醇（B），对血液裂殖体杀虫效果的三批实验平均值。

复方制剂和剂量	复方第一成分的剂量效应（mg/kg/日）	
（mg/kg/日）	ED_{50}	ED_{90}
本芴醇(B)	1.30	2.70
+A 0.25	0.84	1.84
+A 0.50	0.75	1.57
+A 1.00	0.51	1.16
+A 2.00	0.16	0.57
+A 4.00	0.06	0.29
蒿甲醚(A)	2.00	5.30
+B 0.37	1.49	4.46
+B 0.50	0.87	2.67
+B 0.75	0.93	3.44
+B 1.00	0.37	1.21
+B 1.50	0.25	0.83

表内所列复方中组分A和B的各项ED_{90}均在相加线以下，说明呈增效作用。

实施例3:

对疟原虫的杀虫速度是通过动物体内试验而评定的。当实验动物血中疟原虫达到高密度时，给与动物相当于20×ED_{90}的剂量进行灌胃，然后连续观察动物血中疟原虫的下降速度，按给药动物血中原虫下降90%所需时间计算结果，复方是49.7小时，给本芴醇单药的是64.3小时，给蒿甲醚单药的动物血中疟原虫密度未能降至90%而又回升了。

实施例4：

复方中蒿甲醚和本芴醇的最适配比的临床评定：

在动物实验基础上，并参考蒿甲醚与本芴醇单药的临床疗效，对两药的最适剂量比计算结果为1:4-1:6，当选择1:6配比对每一片复方的药片中蒿甲醚和本芴醇分别为20mg和120mg。设1:5和1:6两个给药组病人进行平行对比临床观察，采用“3天4次”疗法，首次服药4片，以后相隔8、24和48小时各服4片，成人服用总量为16片/人，挑选恶性疟疾病人40例，随机分为两个试验组，给药后的观察指标是：①24小时原虫下降率；②平均原虫消失时间；③平均退热时间；④28天治愈率。结果表明：服药后24小时两组的疟原虫下降率分别为96.3%和94.2%；血中疟原虫消失时间分别为34.8小时和36.0小时以及两组病人平均退热时间分别为23.2小时和22.4小时28天时1:5组疟原虫复燃率达20%，1:6组则为0%（该组病人全部治愈）。证明复方中蒿甲醚、本芴醇对人疟治疗的最适配比为1:6。

实施率5：

蒿甲醇—本芴醇的毒理学评价

用1：6配比的蒿甲醚和本芴醇对小白鼠的急性毒性实验测得的半数致死量（LD_{50}）4555mg/kg（灌胃）。按化学毒力学分级标准，该复方中的两药属于低毒级，对大白鼠和Beagle狗进14天毒性试验分设大、中、低三个剂量组，每天给动物口服一次，连续服14天，观察动物食欲和体重，进行血液学和生物指标的测定，并对主要脏器和靶器官进行病理学检查结果：大白鼠的基本安全剂量相当于临床剂量的40倍，Beagle狗的安全剂量相当于人用剂量的50倍，虽然在大剂量组某些动物靶器官（肝和肾）发现部分异常变化，但停药28天以后，均恢复正常。表明增效的复方毒性低，安全范围宽，没有发现不可逆性毒性反应。

实施例6：

增效的抗疟药复方和单药疗效比较，实验选用两组疟疾病人，以“3天4次疗法”口服给药，每组设有恶性疟疾病人各20例，分别比较复方和蒿甲醚—本芴醇

单药疗效，两个单药的服用剂量与本复方中的含量相同，检测指标是：①服药后24小时疟原虫下降率；②病人平均退热时间；③平均原虫消失时间和；④28天治愈率。结果是：给药24小时疟原虫下降率复方为97%，蒿甲醚单药为95.1%，本芴醇为74.5%；平均原虫消失时间：复方为35.6小时，蒿甲醚为38.7小时，本芴醇为68.4小时；平均退热时间：复方为23.8小时，蒿甲醚为19.7小时，本芴醇为40小时；28天治愈率分别为95%，45%和65%，此项试验性治疗方案可以较清晰地说明复方疗效优于单药。

实施例7：

蒿甲醚－本芴醇复方扩大的临床实验

a，按3天4次疗法口服给药，对400例恶性疟疾病人进行治疗观察所得的主要数据为：①平均疟原虫消失时间23.2至41.0小时；②病人平均退热时间为20.4至25.7小时；③28天治愈率平均96.8%。

b，复方对48例间日疟病人采用3天4次疗法，第一次口服4 片，以后每8、24和48小时各服4片，成人总量16片，试服结果：①平均原虫消失时间为22.8±9.5小时；②平均退热时间为13.6±6.9小时；③28天治愈率为91.67%，证明复方对间日疟的疗效非常显著。

实施例8：

复方片剂的制备

本芴醇	120mg
蒿甲醚	20mg
玉米淀粉	100mg
糊精	40mg
吐温－80	0.6mg
15%玉米淀粉糊	适量
硬脂酸镁	3mg

将蒿甲醚结晶、本芴醇结晶分别过100目筛和60目筛，混合本芴醇筛粉，蒿甲醚筛粉，淀粉和糊精，将混合物过40目筛三次。将吐温－80加到淀粉糊中，再与上述配方混合，混合物用湿法制粒，过40目筛，在减压下50－60℃干燥，加入硬脂酸镁后压片。

US

VERIFICATION OF A TRANSLATION

I, the below named translator, hereby declare that:

My name and post office address are as stated below:

That I am knowledgeable in the English language and in the language in which the below identified international application will be filed, and that I believe that the attached English translations of the People's Republic of China patent application 91102575.8 is true and complete translation of the above-identified international application as filed.

Date: August 8, 1991

Full Name of the Translator
(typed or printed): Weijie TANG

Signature of the Translator: 唐伟杰

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Antimalarial Compositions

The present invention relates to a synergistic antimalarial composition, methods of treating malaria by administering that composition, and to a process for the preparation of that synergistic antimalarial composition.

Drug resistant malaria is a serious clinical and public health problem. The malaria parasite Plasmodium falciparum has developed the versatility of evading the effects of standard drugs such as chloroquine either by genetic mutation or by non-genetic adaption methods. The spread of Plasmodium falciparum resistant to chloroquine and other antimalarial drugs is a major challenge to health care programms in tropical and subtropical countries. Therefore, novel pharmaceutical compositions which diminish the resistance against malarial parasites, are needed for successful therapy.

The antimalarial effect of pharmaceutical preparation containing the individual agent benflumetol has been reported in Chemical Abstracts 97:28538 h and 101:136941u. Other compositions contain combinations of known antimalarial agents, For example, the combination of amodiaquine and tetracycline have been used in the clinic [Suphat Noeypatimanond, et al. (1983), Treatment of Plasmodium falciparum malaria with a combination of

amodiaquine and tetracycline in central Thailand, Trans. R. Soc. Trop. Med. and Hyg. 73(3), 338-340]. Recently another antimalarial combination (FANSIMED, mefloquine, pyrimethamine and sulphadoxine) is undergoing clinical trials [Tropical Diseases Research, Seventh Programme Reprot, Chapter 2; Malaria, UNDP World Bank/WHO. Published by WHO, 1985].

The use of combinations of artemisinine, its derivatives and other antimalarial compounds, such as quinine, has been proposed in the Indian Patent Application 26 BOM/87 and the German Patent Application P37 15 378. Also the synergistic effect of a combination of artemisinine and primaquine is known (Wan Yaode, Cang Qizhong, Pharmacy Bulletin, Vol. 16, No. 1, 1981).

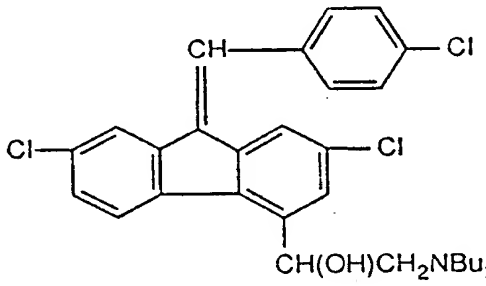
Combinations of the antimalarial agents artemether, arteether, artemisinine dihydro-artemisinine, or artesunate with quinidine or with mefloquine have been disclosed in the European Patent Application 362 810.

Motivation for the present invention has been drawn from the need in therapy for an improved antimalarial composition having higher activity and lower resistance against malarial parasites such as Plasmodium falciparum.

It has now been found that pharmaceutical compositions containing the agent benflumetol in combination with the

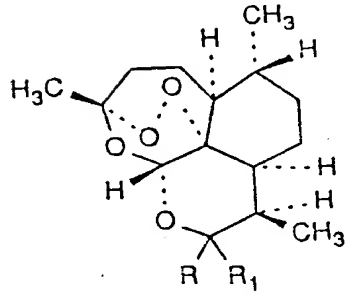
agent artemisinine or especially one of its derivatives such as artemether have excellent antimalarial activity and are more active than compositions containing only the individual component benflumetol, or alternatively, artemisinine derivatives.

The following invention relates to a pharmaceutical composition suitable for synergistic action of the active components against malaria comprising a synergistically effective amount of a compound of the formula:



(I),

combined with a synergistically effective amount of at least one compound of the formula:



(II),

wherein R and R_1 together represent oxygen or one of R and R_1 individually represents hydroxy, C_1-C_6-alkenyuloxy,

C1 -C5 -alkanoyloxy, Carboxy-C1 -C6 -alkanoyloxy, cyclohexanecarbonyloxy, benzoyloxy or naphthoyloxy and the other represents hydrogen. or a pharmaceutically acceptable salt thereof and optionally pharmaceutically acceptable additives.

The general definitions and terms used in this specification of the invention preferably have the following meanings:

The term pharmaceutical composition defines a mixture comprising the compund of the formula I and at least one compound of the formula II. This mixture either consists of a dry preparation of the active components (I) and (II) such as a lyophilisate or preferably contains pharmaceutical carrier suitable for the manufacture of a dosage form such as tablets, capsules or suppositories.

The term synergistic action defines the increase of efficacy of the compositin above the efficacy level of at least one individual active component at the given dose. Preferably, the efficacy of all active components present in the pharmaceutical composition is increased. The synergistic effect is most desirable as it enables the use of a lower dosage of an individual component and/or improvement of activity above the activity levels of the individual components.

The synergism of the claimed composition is proved by experimental results from in-vitro and in-vivo models. The results show that the activity of the component according to formula I is raised as compared to the activity of benflumetol (I) in an individual dosage form and that the acitivity of the component (II) such as artemether is also being raised.

Synergistic action against malaria of the composition according to the present invention permits the combined application of different drug regimens during therapy by the administration of one dosage form such as one or two tablets per day.

The application of a dosage form comprising the active component benflumetol (I) allows permanent action against malaria. The presence in the same dosage form of the second active component (II) such as artemether (R and R' represent methoxy and hydrogen respectively, allows immediate and fast action against protozoa after the outbreak of the disease. These are evident from tests carried out in different standard in-vitro and in-vivo pharmacological models. In the acitve component (I), wherein Bu denotes n-butyl, is known under the name benflumetol, see C.A.R.N. 82186-77-4. Pharmaceutical compositions containing benflumetol individually and their activity against malaria are also known, see the abstacts

according to C.A. 97:28538h and 101:136941. The preparation of benflumetol has been disclosed in the Published Chinese Patent Application 88/076666. X.

The active componet (II) wherein R and R/ together represent oxygen is knwon under the name artemisinine. The component (II) wherein one of R and R/ represents hydrogen and the other represents hydroxy is named dihydroartemisinine.

In a compound of the formula II C/-C6-alkoxy preferably represents methoxy or ethoxy. The compound (II) wherein one of R and R/ represents methoxy and the other represents hydrogen is known under the name artemether. The compound (II) wherein one of R adn R/ represents ethoxy and the other also represents hydrogen is known under the name arteether.

In a compound of the formula II C/-C6-alkenyloxy is preferably allyloxy. C/ -C5-alkanoyloxy is preferably acetoxy or propionyloxy. Carboxy-C -C -alkanoyloxy is preferably carboxy-n-propionyloxy. The carboxy group may be presetn in salt form (carboxylate), e.g. as sodium or potassium salt. The compound (II) wherein one of R and R/ represents sodium carboxylate-n-propionyloxy (-O-CO-CH_2-CH_2-CO_2-Na) and the other represents hydrogen is named artesunate.

The active components artemisinine, dihydroartemisinine, arteether and artesunate comprised by formula II are preferred. Especially preferred is artemether.

The generic names used in the specification of the present invention are taken from "Tropical Diseases Research, Seventh Programme Report", Chapter 2; Malaria, UNDP WORLD BANK/WHO, Published by WHO, 1985.

The active components (II) artemisinine, dihydroartemisinine, arteether, artemether and artesunate are known. Artemisinine has been isolated from Artemisia annual L. and subsequently synthesized. It has been used for the treatment of Falciparum malaria [H.P. Koch (1981) Qinghasosu: a potent antimalarial from plant origin, Pharmacy International (New Drugs), p. 184-185, Elsevier North Holland Biomedical Press; L.J. Bruce-Schwatt (1982), Qinghaosu: a new antimalarial, British Med. J., 184,767-768]. The clinical evaluation of the activity of artemisinine in 2069 patients was reported by Kocvh in 1981, of which 1511 patients were treated for a vivax malaria [H.P.Koch (1981) Qinghaosu: a potent antimalarial from plant origin, Pharmacy International (New Drugs), p. 184-185, Elsevier North Holland Biomedical Press]. It has also been shown to be active against chloroqine-resistant strains of Plasmodium falciparum in man [J.P. Jiang et al.

(1982), Antimalarial activity of mefloquine and qinghasosu. Lancet, ii. 8293, 185-287]. Dihydroartemisinine, arteether, artemether, artesunate are semi-synthetic derivatives of artemisinine. Their antimalarial activity is disclsoed in different WHO reports. [WHO. Report of the Scientific Working Group on the Chemotherapy Malaria, TDR/Chemal 3rd Review, 85.3, Geneva, 3-5. June 1985 and the references contained therein].

The pharmaceutical acceptable carrier used in the present invention are conventional used in the pharmaceutical field. The carrier are used for the preparation of enteral or parenteral dosage forms according to conventional formulaiton methods.

For oral administration suitable carrier include inert diluents of fillers, thereby forming dosage forms such as tablets, powders, capsules, and the like. The pharmaceutical compositions can, if desired, contain additional ingredients such as flavourings, binders, excipients and the like.

For example, tablets containing various solid carrier such as starch, dextrin, alginic acid and certain complex silicates, together with binding agents such as polyvinylpyrrolidone, sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium

stearate, sodium lauryl sulfate and talc are often useful for tabletting purposes. Solid compositions of a similar type may also be employed as fillers in soft and hard gelatin capsules, preferred materials therefore include lactose or milk sugar and high molecular weight polyethylene glycols.

For other oral dosage forms the mixture of the compounds can for example be administered in a gelatin capsule. Such formulation could be based on a suitable refined edible oil such as sunflower oil, peanut oil, coconut oil or til oil.

In a preferred embodiment of the present invnetion, the active components (I) and (II) are formulated in a single unit dosage form such as tablets or capsules.

The active componetns (I) and (II) may also be formulated into two individual dosage forms contained within one administration system (kit of parts), which are simultaneously or consecutively administered. The same route of administration is possible, e.g. administration of two individual dosage forms contained within one administration system (kit of parts), which are simulataneously or consecutively administered. The same route of administration is possible, e.g. administration of two individual dosage forms contained within one kit of

parts. One tablet or capsule containing componet (I) and, consecutively a second dosage form containing component (II) is administered. An individual dose regimen may be developed especially during clinical treamtent, e.g. by administering after the first occurrence of malaria a tablet or capsule containing a high dose of the active component (II) or, correspondingly, multiplying lower doses in the beginning of malaria attacks, and administering also a tablet or capsule containing a lower dose of the active component (I). In the course of treatment, dosage forms containing a lower dose of component (II) and a higher dose of component (II) are adminstered. Different dosage forms present in one kit-of-parts may also be administered simultaneously or consecutively, e.g. by administration of a tablet containing component (I) and a suppository containing component (II). The dosage range may also be varied according to the dose regimens given above.

The usefuleness of the pharmaceutical composition according to the present invention in therapy against malaria is evident from in-vitro and in-vivo results from experiments carried out in established test models. Some results are given in the Examples. The ability of the composition to act as an effective and rapid-acting antimalarial agent even against strains of P. berghei known to be extremely resistant against other antimalarial agents reflects the usefulness of the present invention.

The present invention also relates to a method of treatment against malaria which comprises administering to patient after the outbreak of malaria the above-mentioned pharmaceutical composition comprising the combined active componets (I) and (II). The composition is administered to the patient for a period of time of at least four days, perferably five or more days.

The term method of treatment also comprises prohylactic administration of the composition to healthy patients to prevent the outbreak of the disease in high-risk areas of contamination, especiallyh in regions between the tropics of capricorn and cancer.

The dose of the active component benflumetol (I) as contained in the pharmaceutical composition may vary within wide limits and depends on the condition of the patient and the time period elapsed after the outbreak of the disease. Based on in-vivo data from model experiments as reported below in the Examples, it is established that the daily dose of benflumetol is between about 0.2-5mg/kg, preferably 0.2-10.0 mg/kg and especially about 0.2-5.0 mg/kg. This daily dose can be raised considerably upon need in view of the low toxicity and high tolerability of benflumetol. The component (II) in the composition, especially artemether, is between 0.2 and 5 mg/kg, preferably 0.3-3.0 mg/kg and especially between about 0.4-

5.0 mg/kg.

The dose ratio of component (I) to component (II) may also vary within wide limits. It has been determined that synergism will be especially efficient if benflumetol is administered in equal weight amounts or, preferably, in excess amounts as compared to the weight amounts of component (II) administered. Accordingly, the weight amount of benflumetol may vary from one to ten parts for each part component (II), especially artemether administered. Preferably, three to seven parts and especically five to six parts of benflumetol are administered for each part of component (II). The dose amounts given and dose ratios refer to daily administrations.

The invention also relates to a process for the preparation of the pharmaceutical composition suitable for synergistic action of the active components against malaria which comprises combining an effective amount of a compound of formula I with an effective amount of a compound of the formula II and formulating this combination of active components under optional addition of pharmaceutically acceptable carriers to a suitable dosage form.

The novel pharmaceutical compositions contain, for example, from 10% to 80%, preferably from 20% to 60%, of

the combination of active components. Pharmaceutical compositions according to the invention are suitable for enteral administration and are, for example, formulated into oral dosage unit forms, such as dragees, tables, capsules or suppositories. These are manufactured in a manner known per se, for examples by means of conventional mixing, granulating, confectioning, dissolving or lyophilising processes. For example, pharamceutical preparations for oral administration can be obtained by combining the active ingredient with solid carriers, optionally granulating a resulting mixture, and processing the mixture or granulate, if desired or necessary, after the addition of suitable adjuncts, to form tablets or dragee cores.

In a preferred embodiment of the process, the active components (I) and (II) are milled either individually or together to particle sizes from about 10 to about 400 μ, preferably 20 to 200 μ. The active componetns are present in these ranges.

Particles of this size are obtained by conventional comminution methods, e.g. grinding in an air jet mill, ball mill or vibrator mill. Micronisation is preferably effected by per se by known methods using an ultrasonics disintergrator, e.g. of the Branson Sonifier type as described e.g. in J. Pharm. Sci, 53 (9), 1040-1045 (1965),

or by stirring a suspension with a high-speed agitator, for example with a stirrer fo the Homorex type (supplied by Brogli & Co., Basel). In these preferred methods, micronisation is effected at about 500 to 10,000 rpm by dissolving or suspending the combination of active componets in an organic solvent, e.g. methanol ethanol or propylene glycol, and precipitating it in microcrystalline form at ca. 0 -5° C in water or an aqeous salt solution, e.g. 2% sodium chloride solution which may additionally contain a protective colloid such as gelatin or a cellulose ether, e.g. methyl cellulose or hydroxypropyl methyl cellulose, in low concentration (0.1-1%), and filtering the resulatant stirred suspension. The filter cake is dried at low temperature, e.g. ca. 0 -5° C, under vacuum (e.g. below 50 mbar, preferably at 0.5 mbar). The subsequent drying can be effected at ca. 50 -90° C.

The crystals thus obtained are then formulated to granulates, preferably by wet granulation which is carried out according to standard methods.

The pharmaceutical composition is preferably prepared by compressing a granular formulation which is obtained, for example, by sieving and, if desired, by comminuting the drug, with or without the excipients, compacting with another solvent such as ethanol or water, removing the solvent or drying, with or without the addition of lubricants or glidants such as magnesium stearate or

TWEEN, comminuting the granules and sieving once more.

The granules can be compressed to table cores in a conventional tabletting machine, for example an EKO Korsch eccentric tabletting machine, at a pressure of ca. 10 kN. Coating can effected by applying an aqueous-ethanolic solution in which, for example, polyethylene glycol and saccharose is dissolved or dispersed.

Dragee cores are provided with suitable coatings that may be resistant to gastric juices, there being used, inter alia, concentrated sugar solutions that may contain gum arabic, talc, polyvinylpyrrolidone or polyethylene glycol. Colorings or pigments may be added to doses of active ingrdient.

Further orally administerable pharmaceutical compositions are dry-filled capsules consisting of gelatin, and also soft sealed capsules consisting of gelatin and plasticiser, such as glycerine or sorbitol. The dry-filled capsules may contain the active components in the form of a granulate, for example in admixture with fillers, such as lactose, binders, such as starcches, and/or glidants, such as talc or magnesium stearate, and optionally stabilisers. In soft capsules, the active ingredient is preferably dissolved or suspended in suitable liquids, such as fatty oils, paraffin oil or

liquid polyethylene glycols, to which stabilisers may also be added.

Suitable for enteral administration are also suppositories that consist of a combination of the active ingredient and a suppository base. Suitable as suppository bases are, for example, natural or synthetic triglycerides, paraffins, polyethylene glycols or higher alkanols. It is also possible to use gelatin rectal capsules that contain a combination of the active ingredient and a base material; suitable base materials are, for example, liquid triglycerides, polyethylene glycols or paraffin hydrocarbons.

The following Examples illustrate the invention described above; they are not, however, intended to limit the scope of the invention in an any way.

Examiner 1: Determination of dose ratios for the combination of benflumetol with artemether:

Albino mice were infected with Plasmodium berghei as test strain. By using orthogonal design, parallel contrast experiments were carried out for different doses of the combination according "4-day inhibition test" method. ED_{50} or ED_{90} and the synergistic indices were calculated by means of a linear regression equation.

$$\text{Index of synergism} = \frac{ED_{50} \text{ or } ED_{90} \text{ for individual component}}{ED_{50} \text{ or } ED_{90} \text{ for that component in combination}}$$

Using this equation, the optimal wieght ratio of drugs in this combination aganist malaria is calculated to be 2:0.75 (the indexsynergism for ED 90 > 6). Experiments in the rhesus monkey with Plasmodium Knowlesi were performed and the result showed that the optimal weight ratio of drugs in this combination against malaria is 3-6 parts of benflumetol to each part of artementher.

Example 2: The synergism between the components benflumetol and artemether is determined according to the method of Peters: Am. Trop. Med. Parasitol Vol. 62, pg. 488-492 (1968). The results are reported in the following Table:

Blood schizintocidal action of artemether (A) and benflumetol (B) admimistered orally in varying proportions to mice infected with P. Berghei K/3 N strain in "4-day test" according to Peters (Mean values of three experiments)

Drug and dose (mg/kg/day)	Effective dose of first component (mg/kg/day)	
	ED_{50}	ED_{90}
Benflumetol (B)	1.30	2.70
+ A 0.25	0.84	1.84
+ A 0.50	0.75	1.57
+ A 1.00	0.51	1.16
+ A 2.00	0.16	0.57
+ A 4.00	0.06	0.29
Artemether (A)	2.00	5.30
+ B 0.37	1.49	4.46
+ B 0.50	0.87	2.67
+ B 0.75	0.93	3.44
+ B 1.00	0.37	1.21
+ B 1.50	0.25	0.83

All points representing ED_{50} and ED_{90} of the component (A) and (B) in combination located beneath the addition line synergism between the components.

Example 3: The rate of killing protozoa was determined in-vivo. When the protozoa concentration in the blood of mice increased to high density, a multiple dose equivalent, il.e. 20x ED was given intragastrically.

The rate of decrease of protozoae in blood was observed uninterruptedly after administration. The timespan required for 90% decrease fo the protozoae was 49.7 hours for the combination and 64.3 hours for benflumetol alone. Artemether alone could not kill protozoae to more than 90% before their number increased again.

Example 4: Clinical determination of the best ratio of dose combination between artemether and benflumetol in the combination:

Based on the result of animal experiment with reference to the clinical effective doses of artemether and benflumetol singly, the optimal ratio of dose combination of these two components was calculated to be from 1:4 to 1:6. For example when 1:6 is chosen, the doses of artemether and benflumetol in each tablet would be 20 mg and 120 mg respectively. Two groups of patients given the combination with 1;5 and 1:6 ratios were selected for clinical parallel comparison trials. In both groups, the "3 days and 4 doses" treatment scheme was adopted, i.e. 4 tablets were administered at the first time and then 4 tablets each for three more times with 8,24 and 48 hour intervals. That made altogether 16 tablets for each adult. 40 cases of permicious malaria were selected and divided randomly into two groups. The following parameters were determined in thses two groups after administration: 1) rate for decrease fo protozoae at 24

hours; 2) average time for disappearance of protozoae; 3) average time for subsidence of fever.

4) 28-day cure rate:

The results showed tht at 24 hours after administratin the rates of decrease in protozoa in these two groups were 96.3% and 94.2%, the time periods for disappearance of protozoae were 34.8 hours and 36.0 hours and the average time periods for subsidence of fever were 23.2 hours and 22.4 hours respectively. However, the recrudescence rate on the 28th day in the 1:5 group was 20% while 0% in the 1;6 group (i.e. all of the patients in this group were cured). These results indicate that the optimum ratio of combination of artemether and benflumetol in the combination for treatment of human malaria is 1:6.

Example 5: Toxcological Evaluation of the artemether-benflumetol combination:

The ratio of combination of 1:6 for artemether and benflumetol was used in these experiments. The medium lethal dose (LD 50) for albino mice was found in acute toxicity experiments to be 4555 mg/kg for oral administration. Based on grading criteria for chemical toxicity, this complex prescription is of low grade of toxicity. Toxicity experiments for 14 days were performed in rats and beagles, which were divided into high-,

medium- and low-dose groups. Durgs were administered per os once every day for successive 14 days. Appetite and body weight were observed, hematological and biochemical parameters were determined, and pathological examinations were made in major viscera and target organs of the drugs. The results revealed that the basic safety dose in rats and in dogs was being equivalent to 40-fold and 50-fold the dose used in man respectively. Although some abnormal changes were found in target organs (liver and kindney) in higher dose groups, they recovered to normal on day 28 after administering the last dose. These results indicated that the toxicity of the synergistic combination is low, and the satety range is wide and free from irreversible toxic reactions.

Example 6: Determination of therapeutic effect of individual components as compard to synergisic combination:

Two groups of patients were selected for oral administration and the 3 days and 4 doses treatment scheme. There were 20 patients with permicious malaria in each group. The therapeutic effect of the combination and artemether and benflumetol singly were compared separately. The doses of both drugs in individual administration were about the same as in the complex prescription. The parameters determined were: 1) the rate for decrease of protozoa at 24 h post administrationem; 2) average time for disappearance of protozoae; 3) average

time for fever subsidence and 4) cure rate at the 28th day.

The rates of decrease in protozoae at 24 hours after administration were found to be 97%, 95.1% and 74.5% for the combination, individual artemether, and individual benflumetol respectively. The times for disappearance of protozoae were 35.6 h, 38.7 h and 68.7 h and 68.4h respectively. The average time for fever subsidence was 23.8 h, 19.7 h and 40 h and the 28-day cure rates were 95%, 45% and 65% respeectively. This experimental therapeutic scheme indicated clearly the superiority in therapeutic effect of the combination over the individual drugs.

Example 7: Additional clinical trials for the combination artemether/benflumetol:

a) With the 3 days and 4 doses schemes and oral administration, altogether 400 patients with permicious malaria were treated. Main parametres observed were: 1) average time for disappearance of protozoae (the results were 23.2-41.0; 2) average time for subsidence of fever (20.4-25.7h; 3) 28-day cure rate (average 96.8%).

b) The combination composition was also administered with the 3 days and 4 doses treatment scheme, i.e.4 tablets the first time and then 4 tablets each time at 8,

24 and 48h with a total of 16 tablets for adults. 48 vivax malaria Patients were treated with the combination. The parametres observed were 1) average time for disappearance of protozoa (the results were 22.8±9.5h; 2) average time for subsidence of fever (13.6±6.9h); 3) 28-day cure rate (91.67%). These results demonstrated remarkable therapeutic effect of the combination against vivax malaria.

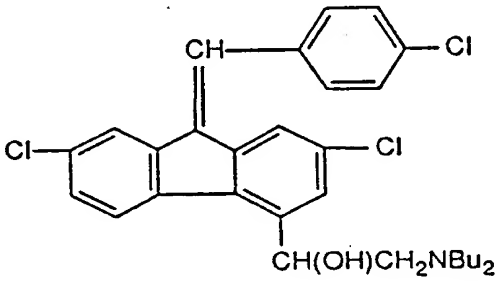
Example 8: Preparation of Tablets.

benflumetol	120 mg
artemether	20 mg
corn starch	100 mg
dextrin	40 mg
Tween®-80	0,6 mg
15 % paste of corn starch	"sufficient"
Mg-stearate	3 mg

Artemether crystals are passed trough a 100 mesh size sieve. Benflumetol crystals are passed through a 60 mesh size sieve and mixed with the artemether solid, starch and dextrin. This mixture is passed 3 times through a 40 mesh size sieve. Tween® -80 is added to the paste of starch which is mixed with the above formulation. This mixture is grnaulated by way of wet-granulation, passed through a 40 mesh size sieve, dried at reduced pressure at 50-60 C. The Mg-stearate is added, and the tablets are pressed.

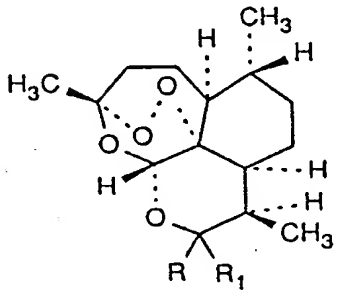
Claims

1. A process for the preparation of a pharmaceutical composition suitable for synergistic action of the active components against malaria which comprises combining a synergistically effective amount of a compound of the formula:



(I),

with a synergistically effective amount of at least one compound of the formula:



(II),

wherein R and R1 together represent oxygen or one of R and R1 individually represents hydroxy, C1-C6 -alkoxy, C1 -C6 alkenyloxy, C1 -C6 - alkanoyloxy, Carboxy-C1-C6 - alkanoyloxy, cyclohexanecarbonyloxy, benzolyloxy or naphthoyloxy and the other represents hyrogen or a pharmaceutically acceptable salt thereof and formulating

this combination of active components under optional addition of pharmaceutically acceptable additives to a suitable dosage form.

2. A process for the preparation of a pharmaceutical composition according to claim 1, which comprises combining a synergistically effective amount of benflumetol (I) with a synergistically effective amount of at least one compound selected from the group consisting of artemisinine, dihydroartemisinine, arteether, artemether and artesunate comprised by formula II.

3. A process for the preparation of a pharmaceutical composition according to claim 1, which comprises combining an effective amount of benflumetol (I) with a synergistically effective amount of artemether comprised by formula II.

4. A process for the preparation of a pharmaceutical composition according to claim 1, which comprises combining one to ten weight amounts of benflumetol (I) with one weight amount of a compound of the formula II.

5. A process for the preparation of a pharmaceutical composition according to claim 1, which comprises combining three to seven weight amounts of benflumetol (I) with one weight amount of a compound of the formula II.

6. A process for the preparation of a pharmaceutical composition according to claim 3, which comprises combining one weight amount of benflumetol with five to six weight amounts of artemether (II).

7. A process for the preparation of a pharmaceutical composition according to claim 1, which comprises formulating the combination of active components under the addition of solid additives to tablets.

FO 7.4/RS/md*

Antimalarial Compositions

Abstract

The invention relates to a synergistic antimalarial composition which comprises the antimalarial agent benflumetol and also an antimalarial agent from the artemisinine group such as artemether. The present invention also relates to a method of treating malaria by administrating said composition and to a process for preparing said composition. The composition can be formulated into solid dosage forms such as tablets and is useful for the treament of drug resistant malaria.